Assiut Vet. Med. J. Vol. 44 No. 88, January 2001

Veterinary Serum and Vaccine Research Institute,
Abbassia, Cairo

EFFECT OF SELENIUM ON THE IMMUNE RESPONSE OF SHEEP VACCINATED WITH PPR VACCINE

(With 3 Tables)

By
I. ISMAIL; A.H. HUSSEIN and A.M. DAOUD

(Received at 19/11/2000)

تأثير السيلنيوم على الإستجابة المناعية للأغنام المحصنة بلقاح طاعون المجترات الصغيرة

ابراهيم إسماعيل ، أحمد حسين ، أحمد محمود داود

تم استخدام عشرة أغنام بلدي قابلة للعدوى بمرض طاعون المجترات الصغيرة في دراسة استمرت ستة أشهر لدراسة تأثير السلينيوم على المناعة العضلية للقاح طاعون المجترات الصغيرة حيث قسمت الأغنام إلى مجموعتين تحوي كل واحدة منها خمسة أغنام ولقد حقنت المجموعة الضابطة تحت الجلد بمقدار ١ ملليتر من لقاح طاعون المجترات الصغيرة الذي يحتوي على 10^3 $TCID_{50}/ml$ بينما حقنت المجموعة المختبرة بنفس الجرعة مضافا إليها ٥ مجم سلينيوم على هيئة صوديوم سلينيت مذاب وقد تم أخذ عينات دم لفصل السيرم على مدار ٢٤ أسبوع حيث استخدمت في قياس تركيز نسبة السلينيوم وكمية الأجسام المناعية إختباري التعادل المصلي والإليزا . وقد أسفرت النتائج عن أن الأغنام المحصنة بلقاح طاعون المجترات الصغيرة قد ظهر لديها نسبةعالية من تركيز السلينيوم وزيادة معنوية في نسبة الأجسام المناعية على مدى مدة التجربة بالمقارنة بالمجموعة الضابطة وبالتالي يمكن القول أن عنصر السلينيوم ثبت له تأثير منشط مناعي للقاح طاعون المجترات الصغيرة في الأغنام.

SUMMARY

Ten Balady sheep susceptible to PPR [Peste des petite ruminant] virus were used in a six-month trial to study the effect of selenium on the humoral immune response of PPR vaccine. They were divided into two groups each of five sheep, control group vaccinated subcutaneously with 1 ml PPR vaccine containing 10^3 $TCID_{50}$/ml, test group vaccinated with 1 ml PPR vaccine added to it 5 mg selenium (as sodium selenite dissolved in the diluent). Blood samples were drown weekly for four

weeks then every two weeks up to 24 weeks post vaccination and assayed for serum selenium concentration, and antibody titres using serum neutralization test and ELISA technique. Compared with those sheep receiving the PPR vaccine alone (control group), sheep received selenium had higher serum selenium concentration and a significant increase in antibody titre during the whole experimental period. Selenium was proved to be immunopotentiating to PPR vaccine in sheep.

Key words: *Selenium, sheep, PPR vaccine, immune response*

INTRODUCTION

Several reports have demonstrated the positive effects of selenium on both the cellular and humoral immune responses of laboratory and farm animals. Spallholz et al. (1973) showed that dietary supplementation with non-toxic levels of selenium led to enhanced IgG and IgM antibody titre in mice, similarly, Berenstein (1978) reported that rabbits given selenium and vitamin E before or during immunization with typhoid vaccine had increased antibody titre, also Bassiouni et al. (1990) found that the immune response of chickens vaccinated with living Newcastle disease vaccine was significantly improved by selenium supplemented in a diet 14 days before vaccination, while Peplowski et al. (1980) reported that selenium supplementation enhanced the immune response of pigs injected with sheep red cells. Subsequently, it was demonstrated that selenium raised the potency of swine fever vaccine (Li and Wang, 1995), but the humoral immune response of horses to tetanus toxoid and equine influenza A virus was increased in selenium and vitamin E supplementation horses (Baalsrud and Overnes, 1986). Selenium injection had improved immunocompetence in cattle as evidenced by the large enhancement of serum IgG titre in response to *Pasteurella haemolytica* vaccination (Droke and Loerch, 1989), to IBR (Reffet et al., 1988 a) and to foot and mouth disease vaccine (Ismail, 1999). Selenium supplementation was important to sheep during pregnancy and lactation as it was essential for growth, fertility, thyroid hormone metabolism and immune function (Kolb et al., 1997 and Wichtel, 1998), selenium supplemented sheep had increased immune response against different pathogen such as tetanus toxoid, parainfluenza-3 virus and Corynebacterium pseudotuberculosis

(Larsen et al., 1988 and Reffet et al., 1988 b), brucella abortus (Jelinek et al., 1988).

This study was performed in order to ascertain if injectable selenium would enhance the primary immune response of sheep vaccinated with PPR vaccine; PPR is a common cause of an acute, highly contagious disease of goats and sheep which possess a serious threat to the development of small ruminants production in several countries including Egypt (Taylor, 1984).

MATERIAL and METHODS

Animals:

Ten Balady sheep 8-10 month old were placed in hygienic house and divided into two groups (each of 5 sheep). They were screened for PPR antibodies by serum neutralization test and proved to be free from antibodies. The animals were free from any stress factors.

Sodium selenite ($Na_2 SeO_3$):

Obtained from Sigma Co., USA.

Vaccine:

Lyophilized PPR vaccine (an attenuated strain of PPR Egypt 87 adopted on vero cells for 25 passages each dose contained 10^3 $TCID_{50}$. obtained from VSVRI, Abbassia, Cairo. Control group (5 sheep) were inoculated with 1 ml PPR vaccine S/C, while test group were inoculated with 1 ml PPR vaccine S/C containing 5 mg as sodium selenite dissolved in the diluent.

Blood samples:

Five ml blood were collected into a sterile MacCartney bottle for separation of serum used for selenium estimation and SNT and ELISA tests.

Methods:

Serum selenium was estimated using atomic absorption spectrophotometer (Perkin Elmer, USA) with lamb for selenium as done by Little et al. (1979).

Humoral immunity:

Serum neutralization test as described by Rossiter and Jessette (1982) in microtitre plates where two fold serial dilution of the pre-inactivated tested serum were done by using Hank's balanced salt solution, serum-virus mixture were incubated at 37°C for 1 hour then assayed on vero cells for 9 days incubation. The neutralizing antibody titers were expressed as the reciprocal of the final dilution of serum in the serum-virus mixture which neutralize an estimated 100 $TCID_{50}$ of

virus at the 50% end point estimated according to method of Reed and Muench (1938).

ELISA test:

A modified method after Anderson and McKay (1994) was used to evaluate antibody titre in Nunc Maxisorb.96 well microtitre plates. Where 50 l of known positive and negative sera, besides tested sera were added at two fold dilution in blocking buffer (PBST + albumin) to the adsorped PPR antigen (containing $10^{2.2}$ $TCID_{50}$/well) and incubated at 37°C for 1 hour followed by 3 times wash with PBS, after the last wash 50 l of anti-sheep immunoglobulin conjugate (horseradish peroxidase) at dilution previously established by titration were added and incubated at 37°C for 1 hour, then washed 3 times with PBS before H_2O_2-OPD was added in phosphate citrate buffer and the color was allowed to develop for 10 minutes, the plates were read on Dynatech MR7000 ELISA reader at an absorbance of 492 nm and the OD values were converted to the anti log_{10}.

Statistical analysis:

Data were analysed using Student (T) test as explained by Snedocor (1969).

RESULTS

1-Effect on serum selenium level:

Table (1) shows that the average level of serum selenium before vaccination did not differ among the two groups but after vaccination serum selenium concentration increased significantly ($p<0.001$ – $p<0.05$) in selenium treated group (test group) during the whole experimental period, this when compared with the values in prevaccination, while in control group serum selenium level decreased but insignificantly for 6 weeks post vaccination.

2-Effect on antibody titer using both SNT and ELISA technique:

Tables (2) and (3) show the effect of selenium on antibody titre using SNT (Table 2) and ELISA technique (Table 3). Test group showed a highly significant increase in antibody titer ($p<0.001 – 0.01$) from the 1st to 24th week post vaccination.

Table 1: Serum selenium µg/dl in sheep vaccinated with PPRvaccine.

Weeks	Control group	Test group
0	8.8 ± 0.17	8.6 ± 0.30
1	8.0 ± 0.24	13.2 ± 0.40 ***
2	8.2 ± 0.21	15.8 ± 0.26 ***
3	8.2 ± 0.09	19.6 ± 0.18 ***
4	8.4 ± 0.23	24.4 ± 0.66 ***
6	8.6 ± 0.30	20.4 ± 0.18 ***
8	8.8 ± 0.26	17.0 ± 0.37 ***
10	8.8 ± 0.38	14.2 ± 0.47 ***
12	8.6 ± 0.30	11.0 ± 0.24 **
14	8.6 ± 0.22	10.0 ± 0.32 *
16	8.8 ± 0.17	10.0 ± 0.28 *
18	9.0 ± 0.20	9.80 ± 0.40 *
20	9.0 ± 0.20	9.80 ± 0.40 *
22	8.8 ± 0.22	9.8 ± 0.22 *
24	8.6 ± 0.30	9.8 ± 0.30 *

* = Significant at p<0.05 **= Significant at p<0.01 ***=Significant at p<0.001

Table 2: Serum neutralization antibody titre expressed by $\log_{10}$ in sheep vaccinated with PPR vaccine (Egypt 87) [X ± S.E., n = 5)

Weeks	Control group	Test group
0	0	0
1	0	0.42 ± 0.03 ***
2	0.42 ± 0.03	0.72 ± 0.03 ***
3	1.23 ± 0.01	1.53 ± 0.03 ***
4	1.53 ± 0.03	1.77 ± 0.01 ***
6	1.47 ± 0.01	1.74 ± 0.02 ***
8	1.44 ± 0.02	1.68 ± 0.01 ***
10	1.38 ± 0.01	1.68 ± 0.01 ***
12	1.32 ± 0.02	1.59 ± 0.02 ***
14	1.23 ± 0.01	1.53 ± 0.01 ***
16	1.11 ± 0.02	1.44 ± 0.02 ***
18	1.02 ± 0.02	1.32 ± 0.02 ***
20	0.96 ± 0.03	1.29 ± 0.02 ***
22	0.84 ± 0.02	1.29 ± 0.02 ***
24	0.72 ± 0.03	1.15 ± 0.02 ***

*** = Significant at p<0.001

Table 3: Serum ELISA antibody titre expressed by log10 in sheep vaccinated with PPR vaccine (Egypt 87) [X ± S.E., n = 5]

Weeks	Control group	Test group
0	0	0
1	0.42 ± 0.03	0.72 ± 0.03 ***
2	0.84 ± 0.02	1.11 ± 0.02 ***
3	1.38 ± 0.01	1.59 ± 0.02 ***
4	1.68 ± 0.01	1.92 ± 0.01 ***
6	1.68 ± 0.01	1.83 ± 0.01 ***
8	1.65 ± 0.0	1.77 ± 0.01 ***
10	1.53 ± 0.01	1.77 ± 0.01 ***
12	1.47 ± 0.01	1.68 ± 0.01 ***
14	1.44 ± 0.02	1.62 ± 0.01 ***
16	1.38 ± 0.01	1.53 ± 0.01 ***
18	1.29 ± 0.02	1.47 ± 0.01 ***
20	1.23 ± 0.02	1.38 ± 0.01 ***
22	1.11 ± 0.02	1.32 ± 0.02 ***
24	0.92 ± 0.03	1.26 ± 0.02 ***

*** = Significant at $p<0.001$

DISCUSSION

The results in Table (1) showed that injection of selenium in a dose of 5 mg/sheep (Blood and Radostits , 1994) caused a significant increase in serum selenium of vaccinated sheep which allowed an increased synthesis of glutathion peroxidase (GSH-PX) and uptake by other body tissues (Hoffman et al., 1978). This prophylactic injection of selenium have been used successfully for prevention particularly in circumstances where diet cannot be easily supplemented or when animals exposed to stress factors like pregnancy and vaccination, also it may be possible to correct deficiency especially our tested sheep that were at lower adequate level of serum selenium (Cohen, 1991).

The slight decrease in serum selenium in control group as a result of stress exerted by vaccination, increased metabolism and breakdown of body tissues that would lead to the production of greater quantities of organic peroxides and free radicals (Fridovick, 1978), the level of glutathione peroxidase would increase to combat these deleterious effects; thus sheep might have a higher demand for selenium at vaccination time.

The results we have obtained confirm the potential immuno-stimulant effects of selenium on immune function as indicated by the significant increase in antibody titer in tested group using both SNT and ELISA tests, our results agree with the studies of Spallholz et al. (1973) and Spallholz et al. (1975) in mice, Berenstein (1978) in rabbits, Sheffy and Schultz (1979) in dogs, Peplowski et al. (1980), Blodgett et al. (1986) and Li and Wang (1995) in pigs, Colango et al. (1984), Bassiouni et al. (1990) and Panda and Roa (1994) in chicks, Baalsrud and Overnes (1986) and Knight and Tyznik (1990) in horses, while Jelinek et al. (1988), Larsen et al. (1988), Reffett et al. (1989 b) in sheep, but Droke and Loerch (1989), Reffett et al. (1989 a), Swecker et al. (1989), Makimura et al. (1993), Nicholesen et al. (1993) and McEvoy and Pollock (1994) in cattle. Selenium has been found to affect lymphocyte subpopulation and in vitro interleukin-2 (IL-2) secretion, both of which are involved critically in B-cell activation, selenium may stimulate B-cell to secrete greater quantities of IgM via selective regulation of lymphocyte subpopulation and lymphokine secretions (Petrie et al., 1989). It may be suggested that the well-known protective role of GSH-PX on membrane integrity might represent at least one of the mechanisms through which selenium enhanced antibody production (Niki et al., 1991).

The synthetic activity of any individual secretory cell (B-cell) is subject to the chemical and physical integrity of endomembranes and the cell surface (Mepham, 1987), selenium as an essential component of the enzyme glutathione peroxidase (GSH-PX) function as an antioxidant by reducing lipid hydroperoxides once formed to less reactive alcohol (Hoekstra, 1975). The oxidative role becomes very important during the immune response when neutrophils produce large quantities of superoxides and hydrogen peroxides from molecular oxygen to destroy ingested foreign organism (Ross, 1977) and so selenium modulate the immune response by protecting lymphocytes from the effects of various inhibitory products produced by phagocytosis (Baumgartner, 1979), also Reffett et al. (1988 a) mentioned that selenium stimulate synthesis of IgM antibody by increasing the number of IgM producing cells. It could be concluded that selenium may stimulate the immune response of sheep to PPR vaccine.

REFERENCES

Anderson, J. and McKay, J.A. (1994): "The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to RP control programe". Epidemiol.Infect., 112:225-231.

Baalsrud, R.J. and Overnes, G. (1986): "Influence of vitamin E and selenium on antibody production in horses". Equine Vet.J., 18(6): 472-474.

Bassiouni, A.A; Zaki, M.M. and Maha, M. Haby (1990): "Effect of vitamin E and selenium on the immune response of chickens against living Newcastle disease vaccine". Vet.Med.J., Giza, 38(1):145-155.

Baumgartner, W.A. (1979): "Antioxidants, cancer and the immune response". Trace metals in health and disease, 295.

Berenstein, J.F. (1978): "Effect of selenium and vitamin E on antibody formation in rabbits". Selenium and Tellurium abstracts, 14:1(abst. 23317).

Blodgett, D.J.; Schuring, G.G. and Kornegay, E.T. (1986): "Immuno-modulation in weanling swine with dietary selenium". Am.J.Vet.Res., 47(7):1517-1519.

Blood, D.C. and Radostits, O.M. (1994): "Veterinary medicine, A textbook of the diseases of cattle, sheep, pigs, goats and horses". 8th Ed., PP.1423.

Cohen, R.D.U. (1991): "Selenium response in unthriftness in sheep and cattle". Cand.Vet.J. 32:113.

Colango, G.L.; Jensen, L.S. and Long, P.L. (1984): "Effect of selenium and vitamin E on the development of immunity to coccidiosis in chickens". Poult.Sci., 63:1136-1143.

Droke, E.A. and Loerch, S.C. (1989): "Effects of parentral selenium and vitamin E on performance, health and humoral immune response of steers new to the feedlot environment". J.Anim.Sci., 67(5):1350-1359.

Fridovich, I. (1978): "The biology of oxygen radicals". Sci.(Washington, D.C), 201:875.

Hoekstra, W.G. (1975): "Biochemical function of selenium and its relation to vitamin E". Fed.Proc., 34:2083.

Hoffman, C.; Rivinus, B. and Swanson, L. (1978): "Effect of intramuscular administration of selenium and vitamin E in dairy heifers on erythrocyte glutathione peroxidase activity and blood selenium levels". J.Anim.Sci., 47:192.

Ismail, I. (1999): "Effect of addition of selenium and/or zinc to FMD vaccine on the immune response of calves". Ph.D.Vet.Med.Sci.Pharmacol., Zag.Univ., Egypt.

Jelinek, P.D.; Ellis, T.; Wroth, R.H.; Sutherland, S.S.; Masters, H.G. and Petterson, D.S. (1988): "The effect of selenium supplementation on immunity and the establishment of an experimental Haemoncous contortus infection in weaner merino sheep fed low selenium diet". Aus.Vet.J., 65(7):214-217.

Knight, D.A. and Tyznik, W.J. (1990): "The effect of dietary selenium on humoral immuno-competence of ponies". J.Anim.Sci., 68(5):1311-1317.

Kolb, E.; Kaskous, S. and Seehawer, J. (1997): "Nutritional aspects of the importance, utilization, metabolism and the use of vitamin E and selenium in sheep". Berl.Munch.Tierarztl.Wschr., 110(5):178-184.

Larsen, H.J.; Moksnes, K. and Overnes, G. (1988): "Influence of selenium on antibody production in sheep". Res.Vet.Sci., 45(1):4-10.

Li, J.X. and Wang, J.D. (1995): "Studies on selenium for raising the immune potency of swine fever vaccine". Acta.Vet.Zootech.Sinica, 26(5):441-445.

Little, W.; Vogg, M.J.; Collis, K.A.; Show, S.R. and Gleed, P.T. (1979): "Concentration of selenium by atomic absorption". Res.Vet.Sci., 26:193-197.

Makimura, S.; Kodama, A.; Kishita, M.; Takagi, H. and Adachi, K. (1993): "Secondary antibody response to Haemophilus somnus antigen in breeding Japanese black cattle fed selenium deficient and alpha tocopherol fortified diets". J.Vet.Med.Sci., 55(5):871-873.

McEvoy, J.D. and Pollock, J.M. (1994): "A preliminary study of peripheral lymphocyte function in cows with chronic endometritis". Vet.Rec., 134:237-238.

Mepham, T.B. (1987): "An analysis of lactation as a productive system". Physiology Lactation, Philadelphia, Open Union Press, 62-68.

Nicholson, J.W.G.; Bush, R.S. and Allen, J.G. (1993): "Antibody responses of growing beef cattle fed silage diets with and without selenium supplementation". Can.J.Anim.Sci., 73(2):355-365.

Niki, E.; Yamamoto, Y. and Komura, E. (1991): "Membrane damage due to lipid oxidation". Am.J.Clin.Nutr., 53:201-205.

Panda, S.K. and Rao, A.T. (1994): "Effect of a vitamin E-selenium combination on chicks infected with infectious bursal disease virus". Vet.Rec., 134:242-243.

Peplowski, M.A.; Mahan, D.C.; Murry, E.A.; Moxon, A.L.; Cantor, A.H. and Ekstram, K.E. (1980): "Effect of dietary and injectable vitamin E and selenium in weanling swine antigenically challenged with sheep red blood cells". J.Anim.Sci., 51(2): 344-351.

Petrie, H.T.; Klossen, L.W. and Kory, H.D. (1989): "Selenium and the immune response: 1-Modulation of alloreactive human lymphocyte function in vitro". J.Leukocyte Biol., 45:207.

Reed, L.J. and Muench, H. (1938): "Simple method of estimating fifty percent end point". Amer.J.Hyg., 27:793-799.

Reffett, J.K.; Spears, J.W. and Brown, T.T.Jr. (1988a): "Effect of dietary selenium on the primary and secondary immune response in calves challenged with infectious bovine rhinotracheitis virus". J.Nutr., 118(2):229-235.

Reffett, J.K.; Spears, J.W. and Brown, T.T.Jr. (1988b): "Effect of dietary selenium and vitamin E on the primary and secondary immune response in lambs challenged with parainfluenza virus". J.Anim.Sci., 66(6):1520-1528.

Ross, D. (1977): "Oxidative killing of microorganisms by phagocytic cells". Trends.Biochem.Sci., 2:61.

Rossiter, P.B. and Jessette, D.M. (1982): "Microtiter techniques for the assay of rinderpest virus and neutralizing antibody". Res.Vet.Sci., 32:253-256.

Sheffy, B.E. and Schultz, R.D. (1979): "Influence of vitamin E and selenium on immune response mechanisms". Fed.Proc., 38(7):2139-2143.

Snedecor, G.W. (1969): "Stastical Methods". 4th edition. The Iowa State Univ.Press, Ames, Iowa, USA. Pp.91.

Spallholz, J.E.; Martin, J.L.; Gerlach, M.L. and Heinzerluig, R.H. (1973): "Selenium: Effect on the primary immune response of mice". Proc.Soc.Exp.Biol.Med., 143:685.

Spallholz, J.E.; Martin, J.L.; Gerlach, M.L. and Heinzerluig, R.H. (1975): "Injectable selenium : Effects on the primary response of mice". Proc.Soc.Exp.Biol.Med., 148(1):37-40.

Swecker, W.S.; Eversole, D.E.; Thatcher, C.D.; Blodgett, D.J.; Schuring, G.G. and Meldrum, J.B. (1989): "Influence of supplemental selenium on humoral immune response in weaned beef calves". Am.J.Vet.Res., 50(10):1760-1763.

Taylor, W.P. (1984): Preventive Vet.Med., 2:157.

Wichtel, J.J. (1998): "A review of selenium deficiency in grazing ruminants: part I new roles for selenium in ruminant metabolism". N.Z.Vet.J., 46(2):47-52.